Review article

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The human Y chromosome

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SUMMARY Despite its central role in sex determination, genetic analysis of the Y chromosome has been slow. This poor progress has been due to the paucity of available genetic markers. Whereas the X chromosome is known to include at least 100 functional genetic loci, only three or four loci have been ascribed to the Y chromosome and even the existence of several of these loci is controversial. Other factors limiting genetic analysis are the small size of the Y chromosome, which makes cytogenetic definition difficult, and the absence of extensive recombination.

Based on cytogenetic observation and speculation, a working model of the Y chromosome has been proposed. In this classical model the Y chromosome is defined into subregions; an X–Y homologous meiotic pairing region encompassing most of the Y chromosome short arm and, perhaps, including a pseudoautosomal region of sex chromosome exchange; a pericentric region containing the sex determining gene or genes; and a long arm heterochromatic genetically inert region.

The classical model has been supported by studies on the *MIC2* loci, which encode a cell surface antigen defined by the monoclonal antibody 12E7. The X linked locus *MIC2X*, which escapes X inactivation, maps to the tip of the X chromosome short arm and the homologous locus *MIC2Y* maps to the Y chromosome short arm; in both cases, these loci are within the proposed meiotic pairing region. *MIC2Y* is the first biochemically defined, expressed locus to be found on the human Y chromosome.

The proposed simplicity of the classical model has been challenged by recent molecular analysis of the Y chromosome. Using cloned probes, several groups have shown that a major part of the Y chromosome short arm is unlikely to be homologous to the X chromosome short arm. A substantial block of sequences of the short arm are homologous to sequences of the X chromosome long arm but well outside the pairing region. In addition, the short arm contains sequences shared with the Y chromosome long arm and sequences shared with autosomes.

About two-thirds of XX males contain detectable Y derived sequences. As the amount of Y sequences present varies in different XX males, DNA from these subjects can be used to construct a map of the region around the sex determining gene. Assuming that XX males are usually caused by simple translocation, the sex determining genes cannot be located in the pericentric region.

Although conventional genetic analysis of the Y chromosome is difficult, this chromosome is particularly suited to molecular analysis. Paradoxically, the Y chromosome may soon become the best defined human chromosome at the molecular level and may become the model for other chromosomes.

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Investigation of persons with abnormal sex chromosome constitutions has led to the inescapable conclusion that, in man, the Y chromosome is sex determining. Independent of the number of X chromosomes present, the Y chromosome induces male differentiation¹⁻³ and the absence of the Y chromosome results in female differentiation.⁴

The Y chromosome differs from the other human chromosomes in two major respects. Unlike the X chromosome and the autosomes, the presence of the Y chromosome is facultative; absence of the Y chromosome results in female development. Also, unlike the other chromosomes, the Y chromosome appears to be bereft of genetic loci. Although the sex chromosomes are thought to have evolved from an identical pair of chromosomes,⁵ the human X chromosome is now approximately three times larger than the Y chromosome.⁶ Over 300 genes and disease loci have been mapped to the X chromosome.^{7,8} In comparison, only a few loci have been assigned to the Y chromosome and, apart from the postulated sex determining gene (or genes), the definition and existence of most of these Y assigned genes is contentious.⁸

The Y chromosome has been largely refractory to classical genetic analysis because its only clearly defined function is in sex determination and because of the lack of recombination. Disruption of the sex determining mechanism leads to loss of the genetic transmission needed for genetic analysis. The need to retain the sex determining gene on the Y chromosome is a strong theoretical reason for the absence of recombination between the sex chromosomes, and the large number of X linked genes not found on the Y chromosome testifies to the absence of extensive sex chromosome recombination.

The limited application and success of the classical techniques for analysing the Y chromosome has posed a challenge to the newer methods of genetic analysis. In the classical approach, the direction of analysis flows from the phenotype to the gene and DNA level. Molecular genetics can be used to reverse this process by first isolating DNA sequences from the Y chromosome and then using these to study phenotypes and function. The small size of the Y chromosome is particularly suited to this type of approach and it is likely that this will be the first human chromosome to be fully defined at the DNA sequence level.

In this review we will compare the Y chromosome structure as deduced from, and predicted by, classical analysis with the Y chromosome as revealed by molecular analysis. Because of the relationship between the sex chromosomes it will be necessary to consider some aspects of the genetics and function of the X chromosome; for a more

FIG 1 The classical model of the Y chromosome (see text).

detailed discussion of the X chromosome the companion review by Dr K Davies in the August issue of the journal should be consulted.⁹ We will also review our own studies with the 12E7 monoclonal antibody which have straddled both the classical and molecular approaches, and finally we will consider briefly the current status of research on the H-Y antigen. The testis determining genes and gene products remain elusive.

The classical model

The Y chromosome can be divided into two major regions of approximately equal size (fig 1); heterochromatic region (Yq11→qter) and euchromatic region (Ypter→q11). The euchromatic region can be further subdivided and the classical model ascribes different functions and properties to the recognisable Y chromosome subregions.

THE SHORT ARM PAIRING REGION

For chromosomal determination of sex it is necessary that the X and Y chromosomes go to different poles during male meiosis and are incorporated into different sperm. Correct segregation of autosomes requires pairing, and cytological observations strongly suggest that in early meiosis pairing occurs between homologous chromosomal sequences.¹⁰ Pairing between the X and Y chromosomes has been

documented in several different mammalian species^{11 12} including humans.¹³ Telomeric association between the short arms of the human X and Y chromosomes can be seen in both light and electron microscopy¹⁴⁻¹⁶ implying sequence homology in this region. Early studies suggested that the pairing, and consequently the homology, involved the distal third of Xp and almost the entire Y chromosome short arm.^{15 16} More recent experiments have shown that the extent of pairing varies extensively between different spreads. Chandley *et al*¹⁷ found that in normal males between 11 and 56% of the Y chromosome could be paired in a synaptonemal complex during early meiotic prophase. The corresponding figures for the X chromosome were between 3.3 and 17.3%. As unpaired chromosomal regions are known to become promiscuous during the progression of meiosis¹⁰ the exact extent of the homologous region is difficult to determine from this type of analysis. It should also be remembered that even the existence of the X-Y homology region is based on assumptions about how chromosomes pair during meiosis.¹⁸

Arguing from the existence of the pairing region and the claim of Hultén¹⁹ that there is an obligatory chiasma for each chromosomal arm in male meiosis, Burgoyne²⁰ has followed Darlington²¹ in proposing that recombination between the X and Y is required for the normal resolution of sex chromosome pairing. If this is the case then the X and Y chromosomes will share sequences by exchange. Depending on the position of recombination events, genes present in the shared region will show only partial sex linkage if occasionally exchanged, or no sex linkage if frequently exchanged. Such genes have been termed 'pseudoautosomal'. Direct evidence for the existence of pseudoautosomal genes has been obtained in the mouse. The dominant mutation *Sxr* causes XX females to develop testes and become infertile males.²² The *Sxr* mutation is a partial duplication of the mouse Y chromosome which results in two copies of the sex determining pericentric region, one of which is translocated to the telomere.^{23 24} During meiosis in the male *Sxr* carriers there is an obligate recombination event which transfers the telomeric copy of the sex determining region to the X chromosome. Thus, male carriers produce equal numbers of four types of gametes containing different sex chromosomes: Y, Y*Sxr*, X, and X*Sxr*. The offspring which receive the Y*Sxr* chromosome will be the new male carriers; the offspring which receive the X*Sxr* chromosome will be sex reversed females. The experimental evidence for X-Y exchange in this system is overwhelming.²⁴⁻²⁶ Indirect evidence for X-Y exchange in normal mice has been described by

Keitges *et al*²⁷ studying the murine steroid sulphatase (*Sts*) locus. However, until direct proof of exchange in normal mice and humans is presented, the conclusion that exchange occurs in the non-pathological case must remain tentative.

THE SHORT ARM SEX DETERMINING REGION
Male sex determination is a result of gonadal sex determination. In the absence of the Y chromosome the gonads become ovaries and in the presence of the Y chromosome the indifferent gonads become testes. The sexual characteristics normally associated with maleness (secondary sexual characters) are a consequence of developing a testis. These observations define the testis determining factors which are deduced to be the product of a Y encoded gene or genes.²⁸⁻³⁰ Applying William of Occam's razor, the simplest explanation is that a single Y linked gene, the testis determining gene, encodes a single polypeptide, the testis determining factor, which induces testicular development. Of course, more complex models can be envisaged and the postulated testis determining factor has not been biochemically isolated or characterised. A brief discussion of the proposed relationship between the testis determining factor and the H-Y serologically determined antigen will be presented at the end of this review.

Karyotypic analysis of Y chromosome structural abnormalities has been used to localise the testis determining gene on the Y chromosome (reviewed in detail by Davis³¹). The conclusion of the majority of these studies is that the testis determining gene is located in the pericentric region of the Y chromosome short arm. This conclusion is also consistent with the karyotype of several XX males who appear to have acquired Yp material^{32 33} on one X chromosome short arm or an autosome.³⁴ However, not all XX males have observable karyotypic additions³⁵ and several patients have been described who apparently contain only Yq material and have testicular elements.^{31 36 37} The apparent contradictions may imply that several regions of the Y chromosome contribute to the normal development of the testis. One model for this proposal might be the X chromosome of *Drosophila melanogaster*. Multiple elements of this X chromosome are involved in setting the X/autosome balance which ultimately regulates sex determination in these flies (reviewed by Baker and Belote.³⁸ See also Sharat Chandra³⁹). Alternatively, the karyotypic interpretations of the human Y chromosome abnormalities may be wrong. Two possibilities for error exist. First, the small size of the Y chromosome makes precise definition of deletions and translocations impossible. Second, unexpected chimaerism may

make the karyotype of the tissue tested, usually blood or skin, different from the karyotype of the gonad.

THE LONG ARM EUCHROMATIC REGION

Deletions of the Y chromosome have also been used to investigate factors required for spermatogenesis. In one well defined case a small deletion of the euchromatic part of the Y chromosome long arm was found in association with azoospermia⁴⁰ and several similar cases have been described.³¹ Again caution must be exercised in accepting this evidence as conclusive.

THE LONG ARM HETEROCHROMATIC REGION

The terminal two-thirds of the Y chromosome is heterochromatic, stains very brightly with quinacrine mustard,⁴¹ and probably does not contain transcribed sequences. This view is supported by the occurrence of healthy, fertile men who show extensive variation in the length of the heterochromatin^{42 43} and by the presence of this region in 1 in 3000 normal females.⁴⁴

In summary, the classical description of the Y chromosome defines four broad regions (fig 1): an X-Y homologous short arm pairing region, containing a subregion of sequence identity if X-Y recombination occurs; a pericentric region on the short arm containing the sex determining gene(s) (see, however, ref 45 and 46 for an alternative view); a euchromatic long arm region containing factors required for spermatogenesis; and an apparently functionless heterochromatic region.

Evidence for homology between X and Y linked expressed genes

The hypothesis that the X and Y chromosomes share genes derives from the sex chromosome pairing hypothesis²⁰ and from phenotypic observations of subjects with Turner's syndrome.⁴⁷ This

hypothesis is supported by our studies with the 12E7 antibody.

The 12E7 monoclonal antibody was raised by Levy *et al*⁴⁸ by immunising mice with lymphoid cells from a patient with T cell acute lymphocytic leukaemia. Similar monoclonal antibodies have been raised by immunisation with red blood cells (BANR; P Goodfellow, P Tippet, and G Banting, unpublished observations), lymphoid cells (RFBI; Bodger *et al*,⁴⁹ P Goodfellow, G Banting, M Bodger, and F Katz, unpublished observations), and melanoma cells (013 and F21; Dracopoli *et al*,⁵⁰ G Banting and P Goodfellow, unpublished observations). The 12E7 antibody defines a cell surface antigen which has a wide tissue distribution in humans.⁵¹ The antibody does not react with mouse cells. This species specificity was exploited by using human-mouse somatic cell hybrids to assign the gene controlling antigen expression to a specific human chromosome.^{52 53} The principle of this approach is simple. Hybrid cells containing both human and mouse chromosomes can be produced by cell fusion. For reasons which are not clear, human-mouse hybrid cells retain a full complement of mouse chromosomes but lose human chromosomes. The presence or absence of a human product can be correlated with the presence or absence of a human chromosome by testing a panel of hybrids containing different human chromosome contributions. The presence of the 12E7 antigen, as measured by indirect radioimmunoassay, correlates with the presence of the human X chromosome.⁵² This was confirmed using hybrids which retained the human X chromosome as the only human chromosomal contribution (table 1). The X linked gene was named *MIC2* in accordance with international rules; later the name was amended to *MIC2X*. The somatic cell genetics approach was extended by studying hybrids retaining only fragments of the X chromosome and the *MIC2X* has been localised to

TABLE 1 Expression of the 12E7 antigen on somatic cell hybrids containing a single human sex chromosome.

	Human chromosome present	*cpm bound $\times 10^{-3}$	
		12E7 (SD)	P3-X63-Ag8 (SD)
Hybrid: MOG13-9†	X	20.4 (0.7)	1.3 (0.1)
Mouse parent: RAG	—	1.3 (0.2)	0.8 (0.1)
Hybrid: HORL9-X‡	X	24.6 (0.9)	0.9 (0.3)
Mouse parent: IR	—	1.8 (0.1)	1.4 (0.1)
Hybrid: 3E7§	Y	34.5 (1.3)	0.7 (0.05)
Mouse parent: RAG	—	0.8 (0.05)	0.8 (0.05)

*The conditions of the binding assay are described in Goodfellow *et al*.⁵² P3-X63-Ag8 is an immunoglobulin class matched negative control. The figures represent the average of three or more determinations.

†Povey *et al*.⁵⁴

‡Goodfellow.⁵⁵

§Marcus *et al*.⁵⁶

the region Xp22.32→pter.^{53 57} This region represents at most a few percent of the X chromosome and lies well within the X-Y pairing region.

The genes *STS* and *Xg* have also been localised to the terminal part of the X chromosome short arm.⁵⁸⁻⁶⁰ The *STS* locus encodes the enzyme steroid sulphatase; deficiency of this enzyme results in X linked ichthyosis.⁶¹ The *Xg* locus encodes the red blood cell antigen Xg^a.⁶² Unlike other X linked genes, *Xg* and *STS* both escape X inactivation.^{62 63} The localisation of the *MIC2X* locus in the same region as *Xg* and *STS* prompted us to test whether *MIC2X* escapes X inactivation. In collaboration with T Mohandas and colleagues we tested a series of human-mouse somatic cell hybrids which contained an inactive X chromosome as the only human sex chromosome contribution. These hybrids expressed the 12E7 antigen proving that, like *Xg* and *STS*, *MIC2X* escapes inactivation.⁶⁴

A gene present on both X and Y chromosomes would be present in two functional copies in males, but, if subject to X inactivation, only present in one functional copy in females. Functional dosage would be maintained if the X linked homologue escaped X inactivation. Although neither *Xg* nor *STS* have known Y homologues, the escape from X inactivation and the regional localisation within the putative pairing region induced us to test for the presence of a Y linked homologue of *MIC2X*. Again we exploited somatic cell genetics; human-mouse hybrids which had retained the human Y chromosome, and no other detectable human genetic material, expressed the 12E7 antigen (table 1).⁵³ This defined the gene *MIC2Y* which is perhaps the first gene assigned to the human Y chromosome. *MIC2Y* was localised on the Y chromosome in the euchromatic region (Ypter→q11).⁵³

In summary, *MIC2X* is an X linked gene which controls expression of the 12E7 antigen. *MIC2X* escapes X inactivation and is localised at the tip of the X chromosome short arm. *MIC2Y* also controls expression of the 12E7 antigen and is localised in the euchromatin of the Y chromosome. *MIC2X* and *MIC2Y* are the first pair of related genes shown to be shared by the sex chromosomes in any mammal.

EVIDENCE FOR BIOCHEMICAL SIMILARITY, OR IDENTITY, BETWEEN THE PRODUCTS OF *MIC2X* AND *MIC2Y*

The simplest hypothesis to explain our results is that *MIC2X* and *MIC2Y* are related structural genes encoding polypeptides which encode the 12E7 antigenic determinant. One alternative hypothesis is that the *MIC2* genes induce expression of genes located elsewhere in the genome. This hypothesis requires the unlikely assumption that the mouse

contains structural genes which are not normally expressed. Perhaps more likely is that the products of the *MIC2* loci cause secondary modification of another molecule to produce the 12E7 antigen. Neither of the alternative hypotheses challenge the existence of the related *MIC2X* and *MIC2Y* genes.

We have attempted to define the products of the *MIC2* loci by biochemical analysis and by gene cloning. The biochemical studies will be described in detail elsewhere and only the conclusions will be discussed here.⁶⁵ The gene cloning experiments are still proceeding and only preliminary results can be reported.

None of the monoclonal antibodies which recognise the 12E7 antigen work well in immunoprecipitation, whereas all of the antibodies work well in western blotting. Cell extracts which have been separated according to molecular size by SDS polyacrylamide gel electrophoresis (PAGE) can be transferred to nitrocellulose paper by applying an electric current. The position of an antigen immobilised on the paper can then be determined by incubating with specific antibody, followed by an anti-immunoglobulin detection reagent. In fig 2 binding of 12E7 antibody to a western blot has been detected by anti-mouse immunoglobulin conjugated with horse radish peroxidase. In the extracts from human cells two bands can be detected by western blot analysis, one at 32 500 daltons and one at 29 000 daltons. In extracts from mouse cells only the lower band is visible. We had not expected to detect any bands in the mouse extract as the 12E7 antibody fails to react with mouse cells in indirect radioimmunoassay or immunofluorescence. This apparent contradiction was resolved by cell fractionation. The lower molecular weight polypeptide is found only in the cytoplasm and the 32 500 dalton polypeptide is present only in the cell membrane fractions. Human-mouse hybrids containing either the X or the Y chromosome expressed both the low molecular weight polypeptide and the larger human specific polypeptide (fig 2). Mixing experiments failed to discern a difference between the X and Y controlled polypeptides. Increased molecular resolution was sought by performing two dimensional PAGE combined with western blot analysis. In this technique the resolving power of PAGE is increased by including isoelectric focusing with separates molecules according to charge. This method also failed to distinguish between the products of the *MIC2* loci. The smaller polypeptide differs in isoelectric point from the larger polypeptide by over 2pH units suggesting that these molecules are probably not directly related.⁶⁵

The biochemical analysis is consistent with the 12E7 antigen being the primary product of the

FIG 2 Western blot analysis of whole cell lysates using 12E7 antibody as a probe. Proteins were transferred from an SDS-polyacrylamide slab gel to a nitrocellulose filter according to Towbin *et al.*⁶⁶ The filter was then blocked as described by Batteiger *et al.*⁶⁷ and after incubation with the 12E7 antibody it was incubated with a horseradish peroxidase conjugated rabbit anti-mouse IgG. Bound antibody was detected by colour development using 4-chloro-naphthol substrate (for more detailed description see Banting *et al.*⁶⁵). Track a=mouse cell line RAG.⁵⁶ Track b='Y only' hybrid 3E7.⁵⁶ Track c='X only' hybrid HORL9X.⁶⁸ Track d=human cell line HEB7A.⁶⁹

MIC2X and MIC2Y loci; however, these experiments are not conclusive. Final proof is only possible from peptide analysis, sequence analysis, or gene cloning experiments.

Using a bacterial expression system we have used the 12E7 antibody to recognise cDNA clones which encode the 12E7 antigen. Preliminary analysis has shown that genes encoding the 12E7 antigen are present in the genome on both the X and Y chromosomes and are very closely related if not identical. In situ hybridisation performed in collaboration with V Buckle and I Craig has localised

the relevant sequences to the short arm of the X and Y chromosomes. Although it should be stressed that these results have yet to be confirmed, they offer very strong support for the contention that the X and Y chromosomes share expressed genes in the pairing region.

Xg AND Yg

The Xg blood group system has several interesting features (for a review see Race and Sanger⁶²). The Xg locus is polymorphic with two alleles: Xg^a, which encodes the Xg^a antigen, and Xg, the product of which has not been defined with antibodies. Female heterozygotes at the Xg locus (Xg^a/Xg) have a single Xg antigen positive population of red blood cells, suggesting that either the Xg locus escapes X inactivation or that Xg expression on red blood cells is extrinsic. The presence of Xg^a antigen positive and negative populations in chimaeric persons strongly supports the suggestion that the Xg locus escapes X inactivation. All of the genetic evidence is consistent with there being a single X linked Xg locus present in the Xp22.3→pter region of the X chromosome.⁶⁰ There is no evidence for a Y linked Xg locus. Expression of the Xg^a antigen on nucleated cells is controversial.^{70 71}

In contrast, the 12E7 antigen is found on nucleated cells as well as red blood cells and is encoded by both X and Y linked genes. Nevertheless, the level of expression of the 12E7 antigen on red blood cells is regulated by the Xg locus and by a related locus Yg, present on the Y chromosome.⁷²

12E7 antigen expression on red blood cells, but not nucleated cells, is polymorphic. Subjects are either 12E7 antigen high level or low level expressors. This polymorphism can be detected both by radioimmunoassay and by indirect haemagglutination. Low level antigen expressors have approximately one-third of the antigen levels of high level expressors. Scoring persons for both the Xg polymorphism and the 12E7 polymorphism revealed an unsuspected relationship between the loci. Xg(a+), antigen positive females express high levels of 12E7 antigen and Xg(a-), antigen negative females express low levels of the 12E7 antigen. In females the level of 12E7 antigen expression can be predicted from the Xg phenotype. Xg(a+), antigen positive males are also 12E7 high level expressors. However, Xg(a-), antigen negative males can express either high or low levels of 12E7 antigen. As summarised in table 2 we suggested that this complex sex limited behaviour was due to a Y linked gene Yg with two alleles: Yg^a associated with high levels of 12E7 antigen expression and Yg associated with low level antigen expression. This hypothesis was confirmed by Tippet *et al.*⁷³ who demonstrated

TABLE 2 Postulated phenotypic relationship between the Xg and Yg locus.

Sex	Genotype	Phenotype	
		Xg antigen	12E7 antigen
Female	Xg ^a /Xg ^a	a+	High
	Xg ^a /Xg	a+	High
	Xg/Xg	a-	Low
Male	Xg ^a /Yg ^a	a+	High
	Xg ^a /Yg	a+	High
	Xg/Yg ^a	a-	High
	Xg/Yg	a-	Low

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that all Xg(a-), antigen negative brothers share the same 12E7 antigen phenotype.

Despite the similarities between the Xg and Yg loci it should be stressed that the Yg locus does not encode the Xg antigen. Similarly the 12E7 antigenic epitope and the Xg antigenic epitope are different. Chimpanzees, gorillas, and man all express the 12E7 antigen; orangutans, gibbons, and all other species tested do not. Only gibbons and man express the Xg antigen.⁷⁴

The Yg locus is the second well defined locus to be assigned to the human Y chromosome and the symmetry between Xg, MIC2X and Yg, MIC2Y is striking. We hope to discover the precise relationship between these four loci by using our molecular probes for MIC2X and MIC2Y.

A molecular description of the human Y chromosome

Just as the cytogenetic map of the Y chromosome has been correlated with subregions defined by classical reasoning, it is also possible to analyse the subregions by molecular analysis. The current map of the Y chromosome as deduced from direct molecular analysis is summarised in fig 3. Cloned sequences which have been assigned to the Y chromosome but have not been formally localised are listed in table 3.

Three main methods have been used for isolating unique sequence probes for the Y chromosome. The most obvious approach is to screen unique sequence DNA, isolated at random from human cDNA or genomic DNA libraries, on male and female genomic DNA. This approach is laborious and not suited to isolating large numbers of probes. Nevertheless, the first probe for a unique sequence shared by the sex chromosomes was isolated by this technique.⁷⁷ A more efficient method is to construct genomic libraries with DNA from Y chromosomes, which have been physically separated from the X and autosomes by the fluorescence activated cell sorter.

FIG 3 A molecular map of the Y chromosome. See text for references to localisation.

This method has proved to be very effective for the X chromosome⁷⁸ and several autosomes,⁷⁹ but has been applied with less success to the Y chromosome.^{80 81} Those Y chromosome banks which have been constructed all suffer from contamination with sequences derived from other

TABLE 3 Y encoded sequences which have not been formally mapped to a subregion of the Y chromosome.

ASSP6			
DXYS2*	(76)	DYSI*†	(497)
DXYS3*	(31)	DYS2*	(53)
DXYS4*	(1)	DYS4*	(36a)
DXYS5*†	(47c)	DYS5*†	(27)
DXYS6*	(16)	DYS6*†	(48)
DXYS7*†	(13d)	DYS8*†	(118)
DXYS8*†	(115)	DYS9*	(40)
DXYS9*	(85)	DYS10*	(GUI)
DXYS10*	(41a)		
DXYS11*†	(52d)		
12fI*			
37C*			
647*†			
17*			

*Data taken from Bishop et al⁷⁵ and Miller et al.⁸ DXY sequences are also found on the X chromosome (Xq13→q22).

†These sequences are found in some XX males and are presumably on Yp: see Bishop et al⁷⁵ and Koenig et al.⁷⁶

chromosomes (C R Muller, 1984, personal communication; H Cooke, 1985, personal communication). Most of the well characterised unique sequence probes for the Y chromosome have been isolated by combining somatic cell genetics and molecular biology. Human-rodent somatic cell hybrids which retain only a limited human chromosomal constitution have been used by several groups to construct partial genomic DNA libraries of particular human chromosomes (for example, Gusella *et al*⁸²). For the Y chromosome libraries, human-mouse hybrids or human-hamster hybrids containing the Y chromosome as the only detectable human chromosomal contribution were used to construct total genomic libraries in cosmids or $\phi\lambda$.⁸³⁻⁸⁶ The total libraries were screened with human repetitive DNA to recognise those clones which were derived from the human Y chromosome. The human derived clones were picked and unique sequence probes subsequently isolated by subcloning. In the future, genomic⁸⁷ and cDNA difference cloning techniques (for example, Hedrick *et al*⁸⁸) will be used for the direct isolation of Y derived sequences and expressed genes.

MOLECULAR ANALYSIS OF THE HETEROCHROMATIC REGION

The best correspondence between the classical map and the molecular map is in the long arm of the Y chromosome (fig 3, table 3). The heterochromatic region which stains brightly with quinacrine is composed largely of two repeat sequences, DYZ1 and DYZ2 (reviewed by Cooke *et al*⁸¹ and Willard⁸⁹). These two sequences together represent at least 50% of the total DNA present in the Y chromosome. The existence of these sequences was first intimated by the work of Cooke and Kunkel *et al* during a search for Y chromosome specific sequences. Kunkel *et al*⁹⁰ hybridised male DNA with a large excess of female DNA to produce a probe specific for the Y chromosome. The probe reacted with DNA from normal males but failed to react with DNA from males carrying a Y chromosome deleted for the heterochromatic region.^{90 91} Cooke applied the more direct approach of cleaving male and female DNA with different restriction enzymes. After cleavage with HaeIII, separation on agarose gels, and staining with ethidium bromide, 3.4 kb and 2.1 kb bands were found in male but not female DNA.⁹² These bands define the 3.4 kb repeat sequence DYZ1 and the 2.1 kb repeat sequence DYZ2. Subsequently, several members of these repeat families have been cloned and studied in more detail. Cooke has estimated that there are about 5000 copies of the 3.4 kb repeat on the Y chromosome, many of these present in tandem

arrays.^{92 93} Although the 3.4 kb repeat structure appears to be restricted to the Y chromosome, sequences cross reacting with DXZ1 exist elsewhere in the genome.^{89 94 95} There is no detectable concentration of these cross reacting sequences on the X chromosome.^{89 93} The amount of the 3.4 kb repeat sequence present in any male is related to the length of the heterochromatin present on the Y chromosome of that male^{91 94} and this localisation to the heterochromatic region (Yq12→qter) has been confirmed by *in situ* hybridisation.⁹⁴

Reassociation kinetics of the 3.4 kb repeat are consistent with considerable sequence variation.⁹⁶ Direct sequence analysis has also indicated the presence of heterogeneity among DYZ1 sequences. Many clones consist of repeats of the pentameric 'core' 5'-TTCCA-3' and other clones contain related but slightly different core sequences. The latter clones show reduced male specificity.^{95 97}

The DYZ2 family may be even more complex than the DYZ1 family. Although originally defined by HaeIII cleavage as a 2.1 kb band, the basic repeat unit appears to be a 2.4 kb repeat arranged in tandem arrays.^{98 99} Sequence analysis of members of the DYZ2 repeat has revealed a complex structure involving both A-T and G-C rich subregions and the presence of an Alu repeat.⁹⁹ However, it is not certain if all DYZ2 repeats have this structure.

In situ hybridisation has localised the majority of DYZ2 repeats to the distal part of the Y chromosome long arm,⁹⁸ although it has been suggested that some of the sequences may lie in the non-fluorescent part of the long arm (Young *et al*¹⁰⁰ quoted in Willard⁸⁹). The presence of specific concentrations of DYZ2 on the X chromosome has been claimed⁹⁸ and refuted.⁸⁹ These conflicting results are undoubtedly due to heterogeneity within the DYZ2 repeat.

Higher primates share DYZ1 and DYZ2; however, only in humans are these sequences concentrated on the Y chromosome.^{98 101} Thus, just based on these repeats, half of the sequences on the Y chromosome are of recent evolutionary origin. A similar observation has been made in the mouse where 30% of the Y chromosome is related to retroviral sequences.¹⁰²

In addition to the DYZ1 and DYZ2 repeats several low copy sequences have been provisionally assigned to the heterochromatic region. A very striking homology between the X and Y chromosomes was found by Cooke *et al*¹⁰³ who cloned a homologous sequence Y2.13 from both the Y and X. No sequence difference was found in a kilobase of compared DNA. *In situ* hybridisation was used to assign this sequence to the long arm of the Y chromosome although the regional localisation to

the heterochromatic region was not conclusive. The X linked sequence was present in Xq24→qter.

Rappold *et al*¹⁰⁴ have also used in situ hybridisation to study two Y derived sequences present in two $\phi\lambda$ clones, YACG35 (defining *DXYZ1*)⁸⁰ and '2.8'.¹⁰⁵ Probes derived from these clones both hybridised to many places in the human genome including the long arm of the Y chromosome. Unfortunately, the specificity of these reactions are not clear as the probes contain repeat elements.

In conclusion, the heterochromatic region of the Y chromosome is composed mostly of the two repeat elements *DYZ1* and *DYZ2*. The presence of some unique sequence DNA in this region seems likely but has not been formally shown. Most of the sequence present in the heterochromatic region is not shared with the X chromosome.

THE EUCHROMATIC REGION OF THE LONG ARM

Several DNA probes have been isolated which react with unique sequences present in the euchromatic portion of the Y chromosome long arm. In our own studies, we described two probes, 116/21 and 69/6, derived from two independent cosmids, 116 and 69 respectively. Initially we assigned these sequences to the heterochromatic region on the basis of reactivity with DNA from somatic cell hybrids carrying different X;Y translocations.^{84 85} However, the amount of euchromatin transferred with the heterochromatin in these translocations was cytogenetically difficult to evaluate and subsequent in situ hybridisation experiments (V Buckle and I W Craig, unpublished observations) localised sequences reacting with probe 69/6 to the euchromatic region just above the heterochromatic region. At high stringency, reaction with probe 69/6 is restricted to males; at very reduced stringency faint bands are occasionally visible on Southern blots of female DNA. Cosmids 69 and 116 both contain extensive regions of single copy sequence; however, we have so far been unable to find any expressed sequences in these clones.

J-L Mandel and colleagues described the presence of a sequence, *DXS31*(MIA), shared by the X and Y chromosomes. This sequence is present at the top of the X chromosome short arm (Xp22.3→pter) and present on the Y chromosome long arm in the euchromatic region just above the heterochromatic region.^{76 106} A similar tentative localisation on the X and Y chromosomes has been described for the sequence *DXS69* (71-7A).¹⁰⁷ However, we have recently mapped the *DXS69* sequence to Xp21→p22.3 on the X chromosome (C Mondello and P Goodfellow, unpublished observations) and a localisation in the heterochromatic region of the Y has not been excluded.

Another sequence known to reside in the proximal part of the Y chromosome long arm was isolated from a $\phi\lambda$ library constructed with DNA from a Y containing human-hamster hybrid.⁸⁶ Again, at low stringency very weak cross reaction was detected with autosomal sequences. At high stringency the probe was Y specific in man and the higher primates (chimpanzee, gorilla, and orangutan), but, in one old world monkey (*Macaca mulatta*), the probe reacted with highly repeated sequences present in males and females.

Actin sequences are also dispersed throughout the genome and found on both the X and Y chromosomes.¹⁰⁸ The sequences on the X chromosome are located in the pericentric region (Xp11→q11) and the Y linked sequences, *ACT2*, have been localised immediately proximal to the heterochromatic region.⁷⁶ Both the X and Y linked actin sequences are pseudogenes (J-L Mandel, 1985, personal communication).

Sequences cross reacting with a probe for the argininosuccinate synthetase gene are also present on many chromosomes including the X and Y chromosomes.¹⁰⁹ The X and Y linked sequences are pseudogenes.¹¹⁰ The Y linked sequence *ASSP6* has not been localised (table 3).

THE PERICENTRIC OR CENTROMERIC REGION

The sequences responsible for centromeric activity in mammalian cells have not been defined. However, in situ hybridisation suggests that alphoid satellite sequences are associated with the centromeric region of all human chromosomes. We have defined the organisation of the alphoid repeat *DYZ3*, present in the Y centromeric region.^{111 112}

Human DNA cleaved with *EcoR1* produces bands of 340 bp and 680 bp which are clearly visible on ethidium bromide stained agarose gels.¹¹³ Sequencing revealed that these bands contained DNA related to the alphoid satellites of the African Green monkey and other primates (reviewed by Singer¹¹⁴). The association of alphoid satellite sequences and the centromere of each human chromosome was demonstrated by in situ hybridisation.¹¹⁵ More detailed investigation, especially of the X chromosome and the Y chromosome (see below), is also consistent with the suggestion that all human chromosomes contain, at the centromere, a tandem array of diverged alphoid sequences. The precise arrangement and degree of divergence of these sequences may be chromosome specific.^{111 116}

The alphoid satellite present on the X chromosome is arranged as a series of 2.0 kb *BamHI* tandem repeats.^{117 118} These sequences have been named *DXZ1*. Up to 10 000 copies of *DXZ1* may be present on the X chromosome. Even at very

reduced stringency DXZ1 probes react only weakly with Y chromosome derived DNA.^{111 117} However, when we screened our cosmid collection derived from the Y chromosome two clones were found which reacted with DXZ1 probes at low stringency.¹¹² These cosmids, 84 and 97, contained rearranged sequences as both inserts were considerably smaller than the 35 to 50 kb needed for packaging DNA into cosmids. This rearrangement is typical of cloned tandem repeats. Probes prepared from 84 and 97 behaved identically, and at high stringency defined a 5.5 kb EcoRI repeat which was Y chromosome specific (fig 4) and present in about 200 copies. Even at high stringency the probes reacted with sequences present on other chromosomes, but these sequences were not arranged as a 5.5 kb EcoRI repeat. As would be predicted probes 84 and 97 only reacted with DNA from the X chromosome at low stringency.

In situ hybridisation at high stringency confirmed the localisation of the sequences defined by 97 to the Y centromere. Reducing the stringency enabled probe 97 to react with the centromeres of all chromosomes, although strongest cross reaction was seen to chromosomes 14, 15, and 22.

Several of the underlying 170 bp and 340 bp repeats present in the 5.5 kb EcoRI repeats have been sequenced and this has confirmed that these sequences are diverged members of the alphoid family. DXZ1, the Y repeat, and the alphoid consensus all showed approximately 70% homology. No areas of particular sequence conservation were detected.

The Y specific 5.5 kb EcoRI repeat structure is not present on the Y chromosome of higher primates other than man and probe 97 reacts equally well with male and female DNA from chimpanzee and gorillas.

Several groups have used the long arm repeat sequences DYZ1 and DYZ2 as probes for antenatal sex diagnosis. One problem with this approach is that occasionally normal males have greatly reduced amounts of long arm heterochromatin and might be expected to lack most copies of DXZ1 and DXZ2. Sex diagnosis employing the Y specific alphoid sequence probes would avoid this drawback.

THE Y CHROMOSOME SHORT ARM

The classical analysis of the Y chromosome implied extensive sequence homology between the short arm of the X and Y chromosomes and that the sex determining gene(s) should be present in the pericentric region. This hypothesis is clearly incorrect in its simplest form, as molecular analysis has indicated a much more complex structure for the Y chromosome short arm. The short arm has been

FIG 4 Hybridisation of human genomic DNA with cosmid Y97 (DY23). Track 1 EMH4, 2X. Track 2 GM1416B, 4X. Track 3 IDF, XY. Track 4 OX, X4Y. The hybridisation was washed to $0.2 \times$ SSC at 65°C and exposed to XAR film for 12 h at -70°C for autoradiography. (Reproduced with kind permission from Academic Press Inc (London) Ltd.)

analysed both directly by the isolation of DNA sequences and indirectly by using these sequences to analyse XX males.

STRUCTURE OF THE Y CHROMOSOME SHORT ARM AS DEDUCED FROM DIRECT ANALYSIS
The first sequence formally assigned to the Y chromosome short arm was isolated by Page *et al*⁷⁷ as a random clone from a $\phi\lambda$ genomic bank. This sequence, DXYS1, recognised a male specific TaqI restriction fragment in addition to fragments shared

by males and females. In situ hybridisation localised the Y sequence to Yp and the X sequence to the long arm region Xq13→q21.¹¹⁹ Both the X and Y sequences are polymorphic and the Y polymorphism is due to an internal duplication of part of the *DXYS1* region (D Page, 1985, personal communication). The X and Y linked sequences show extensive homology and restriction enzyme mapping suggests the sequences match at better than 99% of the residues. The homology region stretches for at least 36 kb and may extend for 200 to 300 kb (D Page, 1985, personal communication). *DXYS1* is also X linked in the great apes (gorilla, chimpanzee, and orangutan) but these species lack the Y linked sequences. This combined with the close sequence relationship implies that a specific translocation between the long arm of the X and the short arm of the Y chromosome occurred during evolution of the human lineage. These results also imply that the Y chromosome short arm is not homologous with the X chromosome short arm throughout its length.

The latter conclusion is also supported by in situ hybridisation of the Y specific probe p50f2 (related to *DYS7*) to Yp.^{8 75} Some probes, isolated at random from a human Y cosmid collection,^{83 120} also react with the Y and autosomal sequences or with the Y, autosomal, and X chromosome sequences. Neither the autosomal localisation, nor regional localisation on the sex chromosomes has been reported for these probes. One member of this group of probes, p12f, defines a Y chromosome polymorphism which shows racial variation.⁷⁵

The origin of the Y chromosome autosomal shared sequences is not clear; if they are clustered on the Y chromosome and on a particular autosome a large Y-autosomal transposition would be predicted. Alternatively, these sequences may be pseudogenes, with an mRNA origin, as has been postulated for both actin and argininosuccinase sequences, which have been located on both sex chromosomes and on several autosomes.^{108 109}

Two other sequences have been localised to the Y chromosome short arm (unpublished results, in collaboration with V Buckle and I W Craig). The first sequence is recognised by the cDNA clone encoding the 12E7 antigen which has been localised to both Xp (Xp22-32→pter) and Yp. This result constitutes the only direct evidence supporting the existence of homology between the sex chromosomes in the pairing region. The second sequence is defined by probe p75/79 which was isolated from cosmid 75 of our Y chromosome cosmid collection. This probe behaves in a manner similar to *DXYS1*. In *EcoRI* genomic digests it defines a male specific restriction fragment as well as an X specific fragment and several fragments shared by the X and Y

chromosomes. In preliminary experiments we had assigned this sequence to the euchromatic part of the Y chromosome (Ypter→q11) and the long arm of the X chromosome (Xq13→qter^{84 85}). These localisations have been refined by in situ hybridisation and somatic cell genetics to Yp and Xq21. As discussed above the homology between Xq13→q22 and Yp may be extensive; in addition to *DXYS1* and cosmid 75 many probes described by Bishop *et al.*^{75 83 120} and St25⁷⁶ may fall into the same homology block. All of the probes which show a high degree of cross reaction between X and Y sequences (at least 10, see Miller *et al.*⁸) map to Xq13→q22. However, formal demonstration that all these sequences also reside on Yp has not been presented.

The usual view of the localisation of the testis determining factor to the pericentric region of the Y chromosome short arm has been challenged by Magenis *et al.*^{45 46} Investigation of prophase banded chromosomes from three XX males and one XY female localised the *TDF* locus to the distal part of the Y chromosome short arm.

STRUCTURE OF THE Y CHROMOSOME SHORT ARM AS DEDUCED FROM INVESTIGATION OF XX MALES

Two hypotheses have been proposed to explain the existence of sterile males with an XX karyotype: either a mutation in a postulated autosomal or X linked gene or genes required for primary sex determination; or inheritance of the paternal sex determining gene in the absence of the complete Y, perhaps as the result of an abnormal exchange between the X and Y during normal pairing.¹²¹ This first hypothesis was suggested particularly to explain families with several XX males.¹²² However, recently three subjects from one such family were shown to have Y derived sequences.¹²³ There is a good animal model for the second hypothesis. The *Sxr* locus is transferred from the mouse Y chromosome to the X chromosome by what appears to be an obligate chiasmata.²⁴ Females receiving an X chromosome carrying the *Sxr* locus are sex reversed phenotypic males unless the *Sxr* locus is inactivated.^{25 26}

Although these hypotheses are not mutually exclusive and may both be correct, recent evidence from the group of M Fellous, C Bishop, J Weissenbach, and colleagues strongly supports the second hypothesis. These authors have shown that about two-thirds of all XX males contain Y derived sequences.^{75 124} Those XX males which fail to react with Y derived probes may have either smaller translocations of Y chromosome material or may be caused by mutation elsewhere in the genome.

According to the French group XX males can be divided into five groups, four of which contain Y derived sequences. The latter four groups are distinguished by reactivity with different DNA probes. Thus, the first group react only with probe p47c (defining *DXYS5*); the second group reacts with probe p47c and probes p50f2 (defining a sequence related to *DYS7*), p52d, and p118; the third group reacts with the above probes plus probe 48; and the fourth group reacts additionally with probe 27. None of the groups react with a probe defining *DXYS1* (J Weisenbach and D Page, 1985, personal communication) and at least one member of the first group reacts with p75/79 (unpublished observations). As discussed previously, p50f2, *DXYS1* probes, and p75/79 all react with sequences on Yp. If it is assumed that XX males are caused by the transfer of the Y located testis determining gene and that the transfer involves a single chromosomal break, rather than a more complicated rearrangement, then the model presented in fig 5 is a plausible representation of the Y chromosome short arm. This model must be regarded as very tentative. Without defined flanking markers the order *TDF*, *DXYS5*, 75/79, *DYS7* cannot be distinguished from 75/79, *DXYS5*, *TDF*, *DYS7* and there is no direct evidence for the relative position of the *MIC2Y* locus (it is placed towards the telomere in accordance with the classical model). Caution must also be exercised in accepting the original assumptions. The generation of XX males frequently results in X chromosome deletions; the frequency of the Xg (a+) phenotype in XX males is close to male level,⁶² strongly suggesting frequent deletion of the Xg locus. Concomitant with this, deletion transfer of the Yg locus may occur^{124a} (P Tippett, 1985, personal communication). It is conceivable that deletion of the X chromosome might result in the uncovering of a recessive X linked mutation which causes sex reversal. XX males occur in most populations with a frequency of about 1 in 20 000.¹²⁵ This frequency may not be incompatible with the generation of XX males by a complex rearrangement of the Y chromosome.

Notwithstanding the caveats, if the model presented in fig 5 is correct, then the short arm of the Y chromosome is a patchwork of sequences some of which are related to the long arm of the X chromosome (*DXYS1*, p75/79), some are Y specific (*DXYS7*), and some are shared by the X and Y chromosomes (12E7 coding sequences).

H-Y antigen

For the past decade the H-Y antigen has been regarded as the key to understanding male sex

FIG 5 A working model for the Y chromosome short arm. The model is based on data in Bishop et al⁷⁵ and Page et al.¹¹⁹ *DXYS7*, *DXYS8*, and *St25* are also found in some XX males.⁷⁶

determination¹²⁶ and claims have been made that H-Y antigen and the testis determining factor are identical (reviewed by Wachtel³⁰). These claims can be disputed.

H-Y antigen was first discovered by skin grafting between mice of the same inbred strain. Grafts exchanged between males or between females were accepted but grafts transferred from males to females were rejected.¹²⁷ These results define a minor transplantation antigen which is present in males and absent from females. The same antigen can also be recognised by killer T cells which are restricted by the major histocompatibility locus (reviewed by Simpson¹²⁸).

A gene controlling expression of the H-Y antigen has been mapped to the mouse Y chromosome. However, there is genetic evidence that the H-Y antigen controlling gene and the testis determining gene, although mapping close together, are not

identical. Sex reversed female mice (XXSxr, see previous section), which have received a small part of the Y chromosome, are H-Y antigen positive. Recently, McLaren *et al*¹²⁹ have described Sxr', a variant of Sxr, which induces sex reversal but does not cause expression of the H-Y antigen. Although it is possible to argue that the H-Y antigenic epitope has been lost from the testis determining factor without disrupting its sex determining function, the simplest conclusion is that the two are not identical.

Transplantation studies to define minor histocompatibility antigens are much more difficult to perform in outbred species like humans than in inbred mice. Nevertheless, the available data suggest that a Y chromosome controlled H-Y antigen exists in man which can be defined by transplantation or MHC restricted killer T cells.^{130 131} However, in these experiments low level killing of female cells was observed.

The cumbersome nature of transplantation assays was a spur to the development of serological methods for defining H-Y antigen.¹³² Unfortunately, it has not been possible to construct a simple reproducible assay which is also quantitative, nor has it been possible to prove that the serological assays define the same molecules as the transplantation assays.¹³³ For this reason a distinction has been made between H-Y antigen and serologically defined H-Y antigen (sd H-Y antigen).¹³³⁻¹³⁵

Books have been written about the intricacies, complexities, and contradictions of sd H-Y antigen testing. Early studies were hampered by the poor quality and limited quantities of the antisera available. This made comparing the results from different laboratories difficult, a problem which was compounded by the large number of different assays used. Surprisingly the availability of monoclonal antibodies has not simplified the assays used nor produced standard serological reagents. Particular reserve is required when considering sd H-Y antigen typing of persons suffering from sexual dysgenesis and until the serology of sd H-Y antigen becomes clearer we prefer to reserve judgement on its putative role.

In recent years a consensus seems to have arisen that H-Y antigen is present in at least some XO human females (reviewed by Andrews¹³³). If this is the case then sd H-Y is not likely to be a product of the Y chromosome.

Conclusions

Molecular analysis offers the promise of uncovering the secrets of the human Y chromosome and providing the first detailed description of any mammalian chromosome at the molecular level.

The major questions yet to be resolved are the nature, number, and precise location of the sex determining genes. The demonstration that some XX males contain Y derived sequences provides an entry point for analysing, and eventually cloning, these genes. Investigation of XY females for microdeletion of the Y chromosome might also be a useful strategy. These experiments will also help to localise the testis determining gene. Classical analysis of translocations had suggested a short arm pericentric localisation.³¹ However, analysis of the translocated sequence in XX males is consistent with a more distal localisation. This latter interpretation has also been suggested by the karyotype studies of Magenis *et al*.^{45 46}

Several other fundamental questions should also be resolved in the next few years. The number of expressed Y linked genes, the nature of meiotic chromosome pairing and its relationship (if any) to X inactivation, and the exchange of sequences between sex chromosomes at meiosis are all problems which can be approached using cloned sequences derived from the human sex chromosomes.

Traditional views had held that the X and Y chromosomes might share sequences by descent.¹²⁶ This view has been challenged by the observation that none of the repeated sequences (DYZ1, DYZ2, or DYZ3) is shared by the X and Y chromosomes and those unique sequences which are shared are usually not found to be Y linked in other mammals. Apart from the sex determining region, the human Y chromosome is apparently a recent creation. Cytogenetic arguments that most of the short arm of the Y chromosome should be homologous to the short arm of the X chromosome are also unlikely to be correct. The Y chromosome short arm contains sequences which are Y specific, sequences shared with autosomes, and an extensive block of sequences shared with the long arm of the X chromosome. The cloned probe for the *MIC2* loci has defined a sex chromosome short arm homology region but this region cannot include the majority of sequences present on the Y short arm.

Further molecular analysis will undoubtedly challenge our other concepts of the Y chromosome.

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